PATENT
Attorney Docket No. 03806.0590-00
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Swen HÖLDER et al.	)	Group Art Unit: 1624
	)	
Application No.: 10/715,556	)	Examiner: Kahsay HABTE
	)	
Filed: November 19, 2003	)	
	)	Confirmation No.: 5066
For: NOVEL PYRIDAZINONE DERIVATIVES AS PHARMACEUTICALS AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM	)	

MAIL STOP AMENDMENT
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Eric Parmantier, declare and state that:

1. I am a French citizen, residing at 53 Boulevard de Reuilly, 75012 Paris, France.

2. I was awarded a Ph.D degree in Molecular and Cellular Pharmacology in Neurobiology at Université Paris VI, France, in 1995.

3. I have been employed by Sanofi-Aventis S.A. (and its predecessor Aventis Pharma S.A.) for three years and I am currently a Research Team Leader in tumor cell biology division of the department of oncology at Sanofi-Aventis S.A. in France. My CV is attached as Exhibit 1.

4. Based on my education and experience, I provide this testimony based on the following experiments, which were conducted by me or under my direct supervision.

INHIBITION OF HELA CELL LINE PROLIFERATION

5. The inhibitory effects of the inventive compounds according to the present invention on HeLa cell line proliferation were evaluated as follows.

6. Materials and methods

6.1. Preparation of samples

10 mM stock solutions of the inventive compounds of Example numbers 34, 175, 190, 217, 219, 220, and 226 according to the originally-filed specification were prepared in DMSO and stored at -20 °C. The inventive compounds were tested at 10 concentrations, i.e., 10 µM, 3 µM, 1 µM, 0.3 µM, 0.1 µM, 0.03 µM, 0.01 µM, 0.003 µM, 0.001 µM, 0.0003 µM. Twenty-fold (i.e., 20X) dilutions (containing 2% DMSO) were made in culture media without serum.

6.2. Cell lines

The HeLa cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were grown as monolayers in Dubelcco's Modified Eagle Medium containing 2 mM L-glutamine, 200 I.U./ml penicillin, 200 µg/ml streptomycin, and supplemented with 10% (v/v) heat inactivated fetal calf serum. Cells were transferred twice a week at 10^5 cells/ml in 75 cm^2 flasks after trypsinisation.

6.3. Experimental methods

Cells in the exponential phase of growth were trypsinised and resuspended in their culture medium at $2.8x10^4$ cells/ml. The cell suspension was distributed in 96 well Cytostar microplates (Amersham) (5000 cells/0.18ml/well). HeLa cells were incubated for 4 hours at 37°C in an atmosphere containing 5% CO_2. [^{14}C]-thymidine (0.1µCi/well; i.e. 10 µl) and 10 µl of each 20X dilution of test compound were added to reach a final concentration of the inventive compound ranging from 10 µM to 0.3 nM in 0.1 % DMSO. The uptake of [^{14}C]-thymidine was measured at 48h using a Microbeta Trilux counter (Wallac).

6.4. Expressison of results and data analysis

The cpm was measured 48h after the inventive compounds had been added to the media, and was then compared to those obtained with 0.1% DMSO in the control wells. Blank wells contained culture media, 0.1% DMSO and 0.1 µCi of [^{14}C]-thymidine.

The percentage of inhibition was calculated for each treated well as follows:

% Inhibition = {1- [(cpm of treated well – cpm of blank well) / (cpm of control well – cpm of blank well)]} x 100

IC_{50} values were obtained as an average from a dose response curve of 10 concentrations from two separate experiments for all tested compounds. The IC_{50} is the concentration of drug that gives half the maximum value of the inhibition. It is determined by non-linear regression analysis and calculated as a concentration at middle of curve.

7. Results

The IC_{50} values of the inventive compounds of Example numbers 34, 175, 190, 217, 219, 220, and 226 for inhibiting HeLa cell line proliferation are shown in Table I below.

Table I

Example number	IC_{50} [nM]
34	8743
175	1901
190	2393
217	9842
219	3234
220	1602
226	808

As shown in Table I, all of the inventive compounds tested had inhibitory effects on HeLa cell line proliferation.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 17. 07. 2006

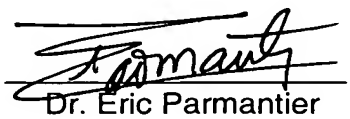
By: 
Dr. Eric Parmantier

EXHIBIT 1
CURRICULUM VITAE

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53, Boulevard de Reuilly
75012 Paris
France
Home : 33 (0)1 43 47 28 41
Mobile : 33 (0)6 83 89 75 77
e-mail: parmantier.el@free.fr

PERSONAL PROFILE

- Qualified molecular and cellular biologist with international academic research experience (7 years)
- Skilled in project and team management
- Has operated as a project leader in R&D in biotechnology (4 years)
- Has operated as a project and team leader in pharmaceutical industry (3 years)

PROFESSIONAL EXPERIENCE

Since 2004 Research Team Leader. Sanofi-Aventis. Oncology. Tumor Cell Biology
Development of receptor tyrosine kinase inhibitors
Development of cell cycle kinase inhibitors
Development of developmental pathways inhibitors

- Management of scientific projects
- Work in project team
- Management of 2 technicians

2003-2004 Research Team Leader. Aventis Pharma. Oncology. Cellular Pharmacology
Development of receptor tyrosine kinase inhibitors
Development of cell cycle kinase inhibitors

- Management of scientific projects
- Work in project team
- Management of 2 technicians

1998-2002 Project Leader and Molecular Biology Manager . Neurotech S.A.
Biotech company developing cell therapies for eye and central nervous system diseases.

- Setting up and organisation of the molecular biology department
- Coordination of technical and scientific activities (team of 6)
- Supervision of 3 technicians
- Conception, optimisation and realisation of expression vectors
- Development and validation of molecular biology tools for the development of therapeutic cell lines
- Involvement in the drafting of preclinical dossiers submitted to regulatory agencies (AFSSAPS, FDA)

1995-1998 Post-doctoral Researcher. University College London, Dpt. of Anatomy and Developmental Biology. Working under Professors R. Mirsky and K .R. Jessen
« Involvement of *Desert hedgehog* during peripheral nerve development »

- First evidence that the protein Desert hedgehog is involved in the differentiation of peripheral nerve connective tissues

1990-1995 Ph.D Thesis. Université Paris VI, Grade-very good. Paper received special praise from the examining jury.
Under the supervision of Dr. B. Zalc. Laboratoire de Neurobiologie Cellulaire, Moléculaire et Clinique (INSERM U134)
"I- Study of HIV-1 gp120 binding on cultured Oligodendrocytes. II- Expression, in the central nervous system, of a peripheral myelin protein : PMP-22"

1993-1995 Molecular biology Teacher. ESTBA

EDUCATION

April 1995 Ph. D. Thesis. Université Paris VI

1989-1990 Post graduate diploma before completing a Ph. D. (DEA) : Molecular and Cellular Pharmacology in Neurobiologie. Université Paris VI

1987-1988 Master degree in Biochemistry and Molecular Virology. Université Paris VII.

AREAS OF SCIENTIFIC EXPERTISE

Neurobiology	Developmental biology of the central and peripheral nervous system Biology of glial cells in the central and peripheral nervous system: Oligodendrocytes and Schwann cells Demyelinating pathologies: Multiple sclerosis, Charcot-Marie-Tooth disease Neurodegenerative pathologies of the central nervous system and of the retina Neuroprotective factors
Angiogenesis	Angiogenic and anti-angiogenic factors Cell therapies of neovascular pathologies of the eye
Cell Penetrating Peptides	Intracellular and nuclear delivery of therapeutic factors using cell penetrating peptides (VP22, Antennapedia, TAT...)
Oncology	Receptor tyrosine kinase inhibitors Cell cycle kinase inhibitors

ADDITIONAL SKILLS

Computer literacy skills	Knowledge of Windows and Macintosh environment : Word, Excel, PowerPoint, ProjectManager Primer Express, Vector NTI, nucleic acid and protein databases
Language skills	French: mother tongue English: TOEIC in October 2002. Level 3/3+ « General Professional Proficiency» (score: 910) German: notions
Project Management	Training: « The key points to project management » (CEGOS)
Intellectual Property	Training: « Introduction to patent » (INPI)

Quality Assurance	Training: « Internal and supplier quality audit » (Sigma Contrôle-Intertek Testing Services) Internal training : « Quality Documentation System » (Neurotech)
Molecular Biology	Training: Q-PCR User ABI PRISM 7000 SDS (Applied Biosystems)

PATENTS AND PUBLICATIONS

PATENTS

2000 Registered a request for a French patent N° 00/07307. « Cellular conditional immortalisation and the protein constructs to put it into use »

PUBLICATIONS

2004 M.B. Alonso, G. Zoidl, C. Taveggia, F. Bosse, C. Zoidl, M. Rahman, E. Parmantier, C.H. Dean, B.S. Harris, L. Wrabetz, H.W. Muller, K.R. Jessen and R. Mirsky. Identification and characterization of ZFP-57, a novel zinc finger transcription factor in the mammalian peripheral nervous system. J. Biol. Chem., **279**, 25653-25664.

2002 R. Mirsky, K.R. Jessen, A. Brennan, D. Parkinson, Z. Dong, C. Meier, E. Parmantier, D. Lawson. Schwann cells as regulators of nerve development. *J Physiol Paris*, **96**, 17-24.

1999 E. Parmantier, B. Lynn, D. Lawson, M. Turmaine, S. Sharghi Namini. L.Chakrabati, A.P. McMahon, K.R. Jessen and R. Mirsky. Schwann cell-derived Desert Hedgehog controls the development of peripheral nerve sheaths. *Neuron* **23**, 713-724.

Mirsky R, Parmantier E, McMahon AP, Jessen KR. Schwann cell-derived desert hedgehog signals nerve sheath formation. *Ann N Y Acad Sci*, **883**, 196-202.

C. Meier, E. Parmantier, A. Brennan, R. Mirsky and K.R. Jessen. Developing Schwann cells acquire the ability to survive without axons by establishing an autocrine circuit involving IGF, NT-3 and PDGF-BB. *J. Neurosci*, **19**, 3847-3859.

Z. Dong, A. Sinanan, D. Parkinson, E. Parmantier, R. Mirsky and K.R. Jessen. Schwann cell development in embryonic mouse nerves. *J. Neurosci. Res.* **56**, 334-348.

1998 N. Spassky, C. Goujet-Zalc, E. Parmantier, C. Olivier, S. Martinez, A. Ivanova, K. Ikenaka, W. Macklin, I. Cerruti, B. Zalc, J.-L. Thomas. Mutiple origin of oligodendrocytes. *J. Neurosci.*, **18**, 8331-8343.

1997 E. Parmantier, C. Braun, J.L. Thomas, F. Peyron, S. Martinez and B. Zalc. PMP-22 expression in the central nervous system of the embryonic mouse defines potential transverse and longitudinal segments. *J.Comp. Neurol.*, **378**, 159-172.

G. Zoidl, A.D. Blanchard, C. Zoidl, Z. Dong, A. Brennan, E. Parmantier, R. Mirsky and K.R. Jessen. Identification of transcriptionnally regulated mRNAs from mouse Schwann cell precursors using modified RNA fingerprint methods. *J. Neurosci. Res.*, **49**, 32-42.

1996 A. Blanchard, A. Sinanan, E. Parmantier, R. Zwart, L. Broos, D. Meijer, C. Meier, K.R. Jessen and R. Mirsky. Oct-6 (SCIP/TST-1) is expressed in Schwann cell precursors, embryonic Schwann cells and postnatal myelinating Schwann cells; comparison with Oct-1, Krox-20 and Pax-3. *J. Neurosci. Res.*, **46**, 630-640.

1995 E. Parmantier, M. Monge, M. Yagello, F. Cabon , C. Demerens, J.C. Gluckman and B. Zalc. HIV-1 envelope glycoptotein gp120 does not bind to galactosylceramide-expressing rat oligodendrocytes. *Virology*, **206**, 1084-1091.

E. Parmantier, F. Cabon, C. Braun., D. D'Urso, H.W. Müller and B. Zalc. Peripheral myelin protein-22 is expressed in rat and mouse brain and spinal cord motoneurons. *Eur. J. Neurosci.*, **7**, 1080-1088.

1993 H. Chen, F. Cabon, P. Sun, E. Parmantier, P. Dupouey, C. Jacque and B. Zalc. Regional and developmental variation of GFAP and actin mRNA levels in the CNS of jimpy and shiverer mutant mice. *J. Mol. Neurosci.* **4**, 1-7.

F. Cabon, E. Parmantier, J. Morser, S. K. Solly, D. Pham-Dinh and B. Zalc. The E. coli envY gene encodes for a high affinity opioid binding site. *Neurochem. Res.* **18**, 795-800.

1992 H. Chen, P. Sun, E. Parmantier, F. Cabon, P. Dupouey, B. Zalc and C. Jacque. Developmental expression of glial acidic protein and actin-encoding messages in quaking and control mice. *Dev. Neurosci.* **14**, 351-356.